Molecular Docking, Quantum Chemical Analyses of Molecular Structure and Spectroscopic Investigations of N-[1-(1-Benzothien-2-yl)ethyl]-N-hydroxyurea: A Combined Experimental and Theoretical Studies

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ABSTRACT

The structure of N-[1-(1-benzothien-2-yl)ethyl]-N-hydroxyurea (C₁₁H₁₂N₂O₂S, Zileuton) was investigated on the basis of spectroscopic data and theoretical calculations. The optimized geometry, intensity and frequency of the vibrational bands of the compound was obtained by Density Functional Theory(DFT) using B3LYP/6-31+G(d,p) basis set. The FT-IR and FT-Raman spectra of the Zileuton were recorded in the range of 4000–400 cm⁻¹ and 4000–400 cm⁻¹, respectively. The harmonic vibrational frequencies were calculated and scaled values have been compared with experimental FT-IR and FT-Raman spectra. The observed and calculated frequencies are found to be in good agreement. A detailed interpretation of the FT-IR and FT-Raman spectra were also reported based on Potential Energy Distribution (PED). To confirm the potential of Zileuton molecule in the area of pharmaceuticals, we have also calculated a series of drug likeness parameters. Possibly important biological activity of Zileuton molecule was also confirmed by molecular docking study.

Key Words: Zileuton, DFT, FT-IR, FT-Raman, Molecular docking

INTRODUCTION

Zileuton's (N-[1-(1-benzothien-2-yl)ethyl]-N-hydroxyurea) mechanism of action in asthma relies on its capacity to inhibit 5-lipoxygenase (in various cell types, specifically macrophages, neutrophils, and eosinophils), the key enzyme that leads to the production of leukotriene A₄ and subsequently leukotriene B₄ (LTB₄), a mediator of inflammation.[1-2]. LTB₄ is an inducer of interleukin-2 (IL-2) and therefore modulates lymphocyte proliferation in response to mitogens[3]. Zileuton also induces certain side effects including nausea, skin rash, diarrhea, insomnia, and elevated liver transaminases but has not been shown to decrease total white blood cell counts[2]. HydroxyUrea (HU), a drug often used to treat

chronic myelogenous leukemia,[4] and reduce the frequency of pain crises and associated complications in patients with Sickle Cell Disease(SCD) in part because of the induction of fetal hemoglobin through nitric oxide production [5-8]. In the present study, the FT-IR and FT-Raman spectral investigation of the Zileuton compound has been performed using the Density Functional Theory(DFT). A complete vibrational analysis of the molecule was performed by combining the experimental and theoretical information using Pulay's DFT based Scaled Quantum Mechanical(SQM) approach. Moreover, the biological activity of the title molecule has been predicted by molecular docking analysis. It is expected that the current investigations might support the development of new potent anticonvulsant agents.

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EXPERIMENTAL DETAILS

The sample Zileuton in the solid form was purchased from the Sigma Aldrich with a purity of greater than 99% and it is used as such without further purification. FT-IR spectrum of the Zileuton compound is measured at room temperature in the region 4000-400 cm^{-1} using BRUKER IFS 66V FT-IR spectrometer equipped with a Global source, KBr beam splitter and MCT detector. FT-Raman spectrum of the sample was recorded using 1064 nm line of Nd: YAG laser as excitation wave length in the region 4000-400 cm^{-1} on a Bruker RFS 100/S FT-Raman spectrometer. The detector is a liquid nitrogen cooled Ge detector. Five hundred scans were accumulated at 4 cm^{-1} resolution using a laser power of 100mW.

COMPUTATIONAL DETAILS

The entire calculations were performed at the DFT (B3LYP) method 6-31+G(d,p) basis set on a personal computer using the Gaussian 03W [9, 10] program package invoking gradient geometry optimization [11]. The optimized molecular structural parameters were used in the harmonic vibrational frequency. The Potential Energy Distribution(PED) was calculated with the help of VEDA 4 software package [12]. Molecular docking (ligand-protein) simulations were performed using AutoDock 4.0.

RESULTS AND DISCUSSION

Molecular geometry

The molecular structure along with numbering of atom of Zileuton is obtained from the Gaussian 09 and GAUSSVIEW program and is shown in Fig. 1. This molecule has nine C-H bond, ten C-C bond, three C-N bond, two C-S bond and one C-O bond.

Vibrational analysis

The Zileuton molecule consists of 28 atoms including oxygen and nitrogen atom. It undergoes 78 normal modes of vibration, and it belongs to the C_1 point group symmetry. The potential energy distribution PED was calculated using the VEDA 4 program, for each normal mode among symmetry coordinates of the molecule. All the assignments are based on the calculated values of Potential Energy Distribution(PED). The recorded FT-IR and FT-Raman spectra are imprinted in Fig. 2 and Fig. 3 along with the computed spectra. All the experimental and theoretical wavenumbers are listed in Table 1.

Figure 1: Optimized structure of Zileuton

O-H vibrations

The O-H stretching band is characterized by very broad band appearing near about 3600-3400 cm^{-1} [13]. Atac et al. [14] assigned O-H stretching at 3584 cm^{-1} using the DFT/B3LYP/6-31+G(d,p) method for Zileuton. This mode 100% pure stretching mode as it is 1311 cm^{-1} in the FT-IR spectra, 1312 cm^{-1} in the FT-Raman spectra and 1330 and 1304 cm^{-1} for the DFT/B3LYP method. The O-H out-of-plane vibration observed at 579 and 584 cm^{-1} for FT-IR and FT-Raman spectra, respectively. 593 and 576 cm^{-1} for the DFT/B3LYP method. The PED for this mode suggests that this is a pure mode with 89% PED.

Figure 2: Observed and simulated FT-IR spectra of Zileuton.

Figure 3: Observed and simulated FT-Raman spectra of Zileuton.

Table 1: Detailed assignments of fundamental vibrations of Zileuton by normal mode analysis based on SQM force field calculations using B₃LYP/6-31+G(d,p) method.

Mode	Observed Frequency		Calculated using B ₃ LYP/6-31+G(d,p) force		Characterization of normal modes with PED ≥10% ^a
	IR	Raman	Unscaled	Scaled	
1			3733	3584	ν OH(100)
2			3623	3478	ν NH _{asy} (100)
3	3451		3599	3455	ν NH _{ss} (99)
4	3112		3221	3092	ν CH (99)
5			3210	3082	ν CH (99)
6		3078	3201	3073	ν CH (99)
7			3191	3063	ν CH (99)
8			3183	3056	ν CH (99)
9		3028	3138	3012	ν CH ₃ (89)
10			3135	3010	ν CH ₃ (88)
11	2992	2990	3116	2991	ν CH ₃ (87)
12		2932	3057	2935	ν CH ₃ (86)
13			1756	1686	ν CC(37), ν NC(18), β HCC(13)
14	1585	1584	1640	1574	NH _{2sc1} (49), ν CN(33)
15	1558	1555	1616	1551	NH _{2sc1} (29), ν CN(25), β CH(15)
16					ν CC _{rock} (68), β CH(18)
17	1517	1520			β CH ₃ (38), ν CC(24)
18					ν CC(33), NH _{2sc1} (15)
19	1450				β CH ₃ (35)
20					β CH(41), ν CC(33)
21					ν CC(27), β HCN(55)
22					β CH ₃ (74)
23					ν CCC(50), β CH(27)
24					ν CC(16), β CHOC(31)
25	1311	1321			ν CC(19), HOC(31), β COC(11)
26					ν CC(77), β CCH(10)
27					β CCH(61), ν CC(23)
28					ν CN(35), ν CC(23), β CH(10)
29	1228	1225			ν CC (43), ν CO(19)
30	1184	1186			rock HCN (66), ν NC(31)
31	1142	1142			ν CN (78), NH _{2rock} (17)
32					β CH(40), ν CC(20), ν CN(10)
33	1109	1113			ν CN(22), ν CC(17), β CH(13), NH _{2rock} (13)
34					ν OC (37), β HCC(20)
35	1082	1088			ν CC (36), β CH(36)
36	1055	1046			ν CO(31), ν CC(27), β CH ₃ (11)
37					β CN(61), β CCN(18)
38					ν NC(13), ν CC (16), β HCC(15)
39					NH _{2rock} (44), ν CN(28)
40		994			ν CN(53), NH _{2rock} (13)
41					ω CH(86), Ring trig (10)

42		967	928	ω CH(88)	67	329	316	β CNC(19), ω NH ₂ (11), ω CCN(10)
43	908	953	915	ω CH(89), Ring trig (10)				
44	854	883	848	ν CO(33), ν CC(30), β CH ₃ (14)	68	292	280	ω NH ₂ (69), ν CNC(12)
45		869	834	τ NCCS(20), τ HCCC(49)	69	244	234	ω NH ₂ (59), ν CNC(11)
46	812 823	856	822	ν CO(12), ν CC(20), β NC(31)	70	221	212	β SC(21), ν OC(18)
47		761	731	τ CN(12), ω OCC(63)	71	211	203	β CSC(12), ω CCC(12)
48	721 727	757	727	τ CN(44), τ NCN (15)	72	196	188	β OC(28), β CCCC(16)
49		748	718	ν SC (28), ω CN(10)	73	156	150	β CCS(10), τ CO(36)
50	701	732	703	ν SC (25), ω CN(12)	74	146	140	τ CH ₃ (37), ω CCH(20)
51	686	716	687	τ CNCN(34), ω SC(21)	75	98	94	β CH ₃ (25), ω CCH(16)
52		687	660	ν SC (25), ω CNC(15)	76	69	66	ν CH ₃ (25), β CH ₃ (21)
53		661	635	ν SC (20), ω CNC(12)	77	38	36	ω CCH(14), β CH(20)
54	584	618	593	rock HOCC(89)	78	28	27	τ CCS(16), τ CO(29)
55	579	600	576	rock HOCC(88)	v; stretching, δ ; in -plane bending, ω ; out-plane bending, ρ ; Scissoring, τ ; rocking , w; weak, m ; medium, vw ; very weak, s ; strong, vs ; very strong, ass ; asymmetric stretching, ss ; symmetric stretching ^a Only PED contributions $\geq 10\%$ are listed.			
56		583	560	β CCN(25), ν CCC(13)				
57		564	541	ν CCC(15), β OCC(36)	NH₂ Vibrations The molecular under investigation possesses one NH ₂ group, the N-H stretching vibrations occur in the region 3500-3300cm ⁻¹ . The asymmetric N-H stretching vibration appears from 3500 to 3420cm ⁻¹ and the symmetric N-H stretching is observed in the range 3420 – 3340cm ⁻¹ [15]. Hence, the bands observed at 3451cm ⁻¹ in FT-IR spectra are assigned to the N-H symmetric stretching vibration. The asymmetric(ν_{asy}) and symmetric (ν_{ss}) N-H stretching mode are calculated by B3LYP/6-31+G(d,p) method at 3478, 3455cm ⁻¹ respectively. The PED for this mode suggests that this is a pure mode, The PED of this mode is 100%. The scissoring mode of the NH ₂ group appears in the region 1615-1650cm ⁻¹ in benzene derivatives with NH ₂ substitutes. The calculated frequency of NH ₂ scissoring mode is at 1574 and 1551cm ⁻¹ for B3LYP/6-31+G(d,p). The experimental band 1584 and 1555cm ⁻¹ in FT-Raman spectra. This is an agreement with the literature values[16,17]. The NH ₂ rocking vibration obtained at 994cm ⁻¹ in FT-IR spectra which are also in agreement with the literature value[18].			
58		548	526	β SC(11), τ CCN(12), ω CCC(12)				
59		533	512	β CN(11), τ CCN(10), ω CCC(10)				
60		494	474	β CO(27), β OCC(20)				
61		482	463	ω CS(11), β CN(23)				
62		449	431	β NCC(15), β CCC(35), β CO(10)				
63		418	401	β CCN(41), τ CH ₃ (12)				
64		401	385	τ CH ₃ (47), β CCN(11)				
65		383	368	τ CH ₃ (22), β COC(21)				
66		364	349	ω NH ₂ (22), τ CN(17), β CN(11)				

C-H vibrations

The hetero aromatic structure shows the presence of C-H stretching vibration in the region 3100-3000 cm^{-1} which is the characteristic region for the ready identification of C-H stretching vibration[19]. In this region, the bands are not affected appreciably by the nature of substituents. In our present work, the C-H stretching vibration was observed at 3114 cm^{-1} in FT-IR spectrum, whereas in the FT-Raman, it is assigned at 3078 cm^{-1} . The scaled vibrations calculated at 3081, 3073, 3064 and 3056 cm^{-1} by B3LYP/6-31+G(d,p) method. As showed by the PED, these modes involve approximately 99% contribution suggesting that they are pure C-H stretching modes. The aromatic C-H in-plane bending vibration occurs within the region 1300-1000 cm^{-1} and out-of-plane bending vibrations in the region 1000-700 cm^{-1} [19]. The C-H in-plane bending vibration computed at 1134, 1109, 1104 and 1098 cm^{-1} by B3LYP/6-31+G(d,p) method shows an excellent agreement with FT-IR bands at 1109 and 1082 cm^{-1} and FT-Raman band at 1113 and 1088 cm^{-1} . The band observed at 908 cm^{-1} in FT-IR and at 854 cm^{-1} in FT-Raman is assigned to C-H out-of-plane bending vibration for Zileuton. This also shows better agreement with theoretical scaled harmonic wavenumber by B3LYP method. The PED corresponding to this vibration is a mixed mode with C-C stretch with a contribution of $\sim 50\%$.

Methyl group vibration

The CH stretching in CH₃ occurs at lower frequencies than those of the aromatic ring (3000–2800 cm⁻¹) [20]. In the present work, CH₃ asymmetric stretching is found at 2992 cm⁻¹ in FT-IR and 3028, 2990 and 2932 cm⁻¹ in Raman spectrum. The CH₃ symmetric stretching is found at 2932 cm⁻¹ in FT-Raman. For methyl substituted benzene derivatives the symmetric bending deformations and rocking modes normally appear around 1465–1440 cm⁻¹ and 1040–990 cm⁻¹ respectively [21]. In the present investigation a strong peak at 1450 cm⁻¹ in FT-IR is assigned to symmetric bending deformation. All these frequencies are shifted towards the maximum. This implies, the methyl vibrations are favored by the presence of NH₂ in the ring. These observations agree well with the earlier work [22, 23]. Also all the above observed frequencies coincide very well with the calculated frequencies.

MOLECULAR DOCKING STUDY

High resolution fine structure of Zileuton (PDB ID: 2O2U) [24] was used as a target protein and AutoDock 4.0 software package [25] was utilized to perform docking simulation study. Initially, hydrogen atoms were added to the target protein and thereafter Kollman atomic charges were calculated and Lamarckian Genetic Algorithm (LGA) was utilized for molecular docking calculations implemented in AutoDock 4.0 software package. The binding pocket of the target pro-

tein was defined using grid size of $80 \times 80 \times 80$ Å with the aid of Autogrid [26].

Figure 4: Summary of docked pose of compound Zileuton, (A) Hydrogen bond interaction (B) 2D Diagram.

The best lowest binding energy of the Zileuton compound to the target protein 2O2U was -6.2 kcal/mol. The amino acid residues involved in hydrogen bonding formation with the title molecule were illustrated using Discover studio Visualizer 4.1 software [27]. The green dotted lines in Fig. 4 denote the formation of hydrogen bonding between the ligand and the target protein in which SERA 146 and ASN A 105 were interacted with the oxygen and nitrogen atoms in the Zileuton compound, respectively. The molecular docking study revealed the possible orientation of the Zileuton molecule inside the binding pocket of its target protein 2O2U.

CONCLUSIONS

The study contained an experimental (FT-IR, FT-Raman) and theoretical (DFT) calculations on the Zileuton molecule. The Zileuton molecule was fully optimized to determine stable structure and have geometric parameters, by using B3LYP/6-31+G(d,p) level of theory. FT-IR and FT-Raman measurements helped functional group identification of the Zileuton molecule. The fundamental wavenumbers are in good agreement with the theoretical results. Molecular docking results are also reported.

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